MOLECULAR EPIDEMIOLOGY of HIV-1 TRANSMITTED DRUG-RESISTANCE AMONG SUBTYPES CIRCULATING in ITALY

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BACKGROUND

- Transmitted drug resistance (TDR) may impair effectiveness of antiretroviral therapy.
- Different HIV-1 subtypes may have different pathways and genetic barrier to resistance to specific antiretrovirals.
- In several western European countries (including Italy) in which an increase in non-B subtypes has been reported, different frequencies of TDR have been observed over time.
- This study aimed to characterize HIV-1 subtype circulation and TDR in Italy using next-generation sequencing (NGS).

METHODS

- Routine plasma HIV-1 RNA NGS genotyping data were collected from newly diagnosed individuals over 2021-2024 retrieved at laboratory and clinical centers partecipating in the Italian collaborative HIV NGS Network created within the ARCA cohort (https://db.dbarca.net/) to share information, protocols and data for harmonizing HIV-1 NGS in the diagnostic setting.
- TDR to PI, NRTI, NNRTI and INI, and genotypic susceptibility were evaluated through HIVdb (ver.9.8) with NGS set at 10% and 20%.
- Subtype and transmission clusters (TC) were determined through the Maximum Likelihood phylogeny based on the GTR+F+R9 model with 1000 replicates fast bootstrapping. Transmission clusters were defined if supported by an intra-genetic distance ≤0.015 and a bootstrap =100.

RESULTS

Table 1. Participant's characteristics at NGS-GRT

Variables	Overall (N=473)
Male, n (%)	328 (69.3)
Age, median (IQR) year	41 (31-51)
Geographic Area, n (%)	
Northern Italy	376 (79.5)
Central Italy	32 (6.8)
Southern Italy	65 (13.7)
Plasma HIV-1 RNA, copies/mL	
<10,000	48 (10.1)
10,001-100,000	148 (31.3)
100,001-1,000,000	186 (39.3)
>1,000,000	91 (19.2)
CD4 cell count, cells/mm^3	
<200	171 (36.2)
201-350	70 (14.8)
351-500	71 (15.0)
>500	80 (16.9)
Unknown	81 (17.1)
Inclusion in TC, n (%)	
In cluster within same center	72 (15.2)
In cluster among different centers	18 (3.8)
Out of cluster	383 (81.0)
Type of TC, n (%)	
Pairs (2 sequences)	54 (11.4)
Small (3-8 sequences)	27 (5.7)
Large (≥8 sequences)	9 (1.9)

RESULTS

More than half of participants (56.2%) harboured HIV-1 non-B strains (Figure 1). **TDR** prevalence to any class was **12.3%** at Sanger like NGS-setting **(>20%)** and slightly increased **(15.9%) at 10% setting**. Regardless of NGS-settings, NRTI-TDR and NNRTI-TDR was below 8%, PI-TDR was low (around 2.5%), and INI-TDR was very low (<0.5%). **TDR was higher in B compared to non-B subtypes, particularly for NRTI (**P<0.05, **Figure 2).**

Figure 1. HIV-1 subtype distribution

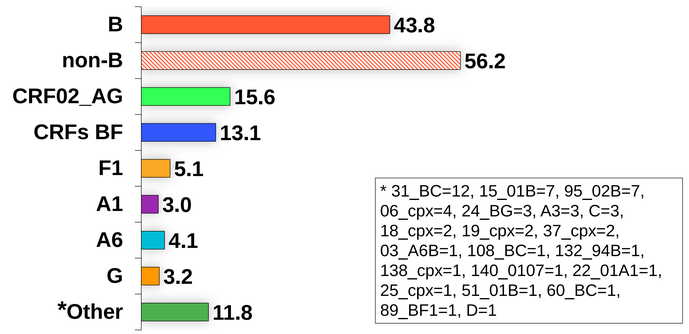
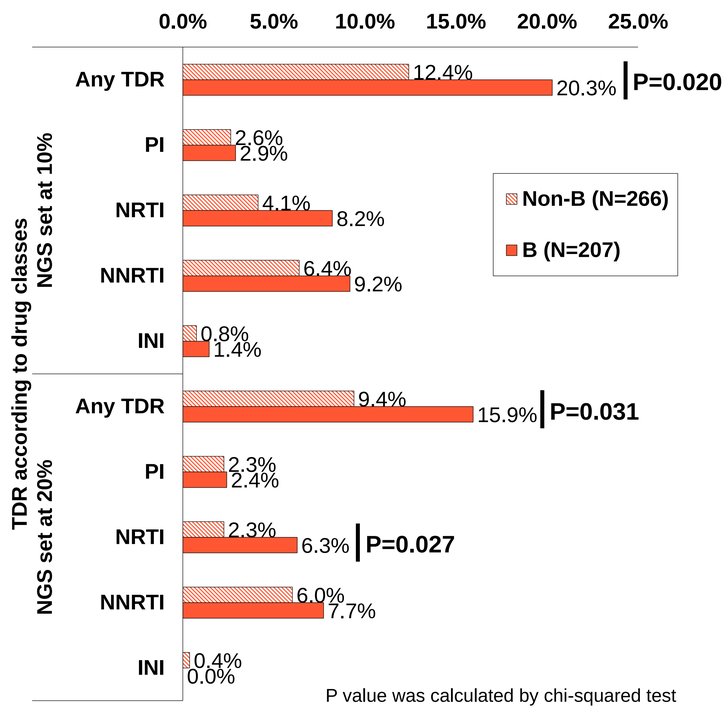


Figure 2. Prevalence of HIV-1 TDR according to subtype



P value was calculated by chi-squared test

Table 2. Genotypic susceptibility of currently approved first-line regimen

Regimen	NGS set at 10%			NGS set at 20%		
	B	Non-B	Pvalue	B	Non-B	Pvalue
BIC+FTC+TD(A)F	206 (99.5)	265 (99.6)	0.859	207 (100.0)	265 (99.6)	0.859
DTG+3TC	207 (100)	266 (100)	0.859	207 (100.0)	266 (100.0)	0.859
DTG+ABC+3TC	206 (99.5)	265 (99.6)	0.859	207 (100.0)	265 (99.6)	0.859
DTG+FTC+TD(A)F	206 (99.5)	265 (99.6)	0.859	207 (100.0)	265 (99.6)	0.859
RAL+FTC+TD(A)F	202 (97.6)	253 (95.1)	0.163	**207 (100.0)**	**254 (95.5)**	**0.002**
DRV+FTC+TD(A)F	205 (99.0)	265 (99.6)	0.422	206 (99.5)	265 (99.6)	0.859
DOR+FTC+TD(A)F	202 (97.6)	260 (97.7)	0.909	204 (98.6)	261 (98.1)	0.719
RPV+FTC+TD(A)F	**178 (86.0)**	**247 (92.9)**	**0.014**	**182 (87.9)**	**249 (93.6)**	**0.031**

The **currently recommended first-line regimens** based on high genetic barrier drugs showed **full activity in >97%** of individuals, regardless of NGS-setting and subtypes. These **proportions were lower** for **rilpivirine- (≥86%)** and **raltegravir-based (≥95%)** regimen regardless of NGS-setting. Rilpivirine susceptibility was lower in the B-subtype subset, while raltegravir was predicted less active in non-B subtypes.

CONCLUSIONS

- A high proportion of HIV-1 non-B subtypes circulate in Italy.
- TDR prevalence is around 16% using NGS set at 10%.
- However, the impact of the detected TDR on the susceptibility to currently recommended first-line regimens is negligible.
- NGS genotyping can improve the characterization of transmission networks of resistance, allowing the detection of TC harbouring minority-resistant species not detectable with Sanger.

Cluster analysis

A total of 35 TC were detected: **27 pairs**, **7 small clusters** (3-8 sequences), and **1 large cluster** (9 sequences). Among them, 23 were found in non-B subtypes (60%). **Six TC had RTI-TDR including**: 3 subtype B and 2 CRF02_AG TC with thymidine analogue mutations; 1 CRF31_BC TC with the NNRTI mutation K103N. Of note, **TDR was detected as minority mutations in three out of six TC**.

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